

1                   38. (Amended) The plant according to claim 32, wherein the promoter induced by  
2 stress is the promoter of the tobacco PR-1a gene.

1                   39. (Amended) The plant according to claim 32, wherein the expression cassette  
2 further comprises the terminator of the tobacco PR-1a gene operably linked downstream of the DNA  
3 sequence encoding the member of the sarcotoxin 1 family or homolog thereof.

### REMARKS

#### Status

Claims 21-26, 29-36, and 38-41 are pending in this application, no claims being added, claims 27, 28, 37, and 42-47 being canceled and claims 21, 23-26, 29-32, 34-36, 38, and 39 being amended herein. Claim 21 is amended to replace the phrase "an anti-bacterial peptide from a Diptera insect" with "a member of the sarcotoxin 1 family or homolog thereof" and to recite the components of the recombinant vectors used to generate the claimed transgenic plants. Support for these amendments may be found in the specification at least on page 2, lines 9-18; page 10, line 24 - page 11, line 3; and page 11, line 32 - page 12, line 33. Claims 23-26, 30, 32, 34-36 and 39 are also amended to replace the phrase "an anti-bacterial peptide from a Diptera insect" with "a member of the sarcotoxin 1 family or homolog thereof". Support for these amendments may be found in the specification at least on page 2, lines 9-18. Claim 24 is also amended to correct antecedent basis. Claim 25 is also amended to correct an obvious misspelling of chitinase. Claim 32 is also amended to recite "promoter induced by stress" instead of "inducible promoter". Support for this amendment may be found in the specification at least on page 9, lines 6-8. Claims 29, 31 and 38 are amended to correct dependencies resulting from canceled claims. Claims 21 and 32 are also amended to replace "the plant before transformation" with "a corresponding untransformed plant" as suggested by the Examiner. No new matter is added by these amendments.

Claims 21, 22, 24-33, and 35-47 were rejected under 35 U.S.C. §112, first paragraph on the grounds that Applicants did not have possession of the claimed invention at the time of filing the application. Claims 21, 22, 24-33, and 35-47 were rejected under 35 U.S.C. §112, first paragraph as allegedly not enabled. Claims 21-47 were rejected under 35 U.S.C. §112, second paragraph on the grounds they are indefinite. Applicants respectfully traverse these rejections.

**35 U.S.C. §112, first paragraph, possession:**

Claims 21, 22, 24-33, and 35-47 were rejected under 35 U.S.C. §112, first paragraph on the grounds that they contain subject matter which was not described in the specification in such a way as to reasonably convey to one of skill in the art that the inventors, at the time the application was filed, had possession of the claimed invention. In particular, the Examiner alleges that Applicants have not satisfactorily described the claimed genus of nucleic acids required to practice the claimed invention. Applicants respectfully disagree.

Recently, the Court of Appeals for the Federal Circuit (“CAFC”) stated that “[i]n written description cases, ‘the primary consideration is factual and depends on the nature of the invention and the amount of knowledge imparted to those of skill in the art by the disclosure.’” *Union Oil Co. v. Atlantic Richfield Co.*, 208 F.3d 989, 996 (Fed. Cir. 2000), quoting *In re Wertheim*, 541 F.2d 257, 262 (CCPA 1976). The CAFC also stated:

**The written description requirement does not require the applicant ‘to describe exactly the subject matter to be claimed,** instead the description must clearly allow persons of ordinary skill in the art to recognize that he or she invented what is claimed.” [emphasis added] *Id.* at 997, quoting *In re Gosteli*, 872 F.2d 1008, 1012 (Fed. Cir. 1989).

The CAFC further stated:

Rather, the Patent Act and this court’s case law require only sufficient description to show one of skill in the refining art that the inventor possessed the claimed invention at the time of filing.” *Id.*

Here, the Applicants amend claims 21, 23, 25, 26, 30, 32, 34-36 and 39 herein to replace “anti-bacterial peptide from the Diptera insect” with “member of the sarcotoxin 1 family or homolog thereof.” These amendments clarify the scope of the claimed invention. Applicants claim:

A method of conferring resistance to pathogenic fungi on a plant using a DNA sequence encoding a member of the sarcotoxin 1 family or homolog thereof, the method comprising the steps of: transforming a plant cell by introducing the DNA sequence encoding the member of the sarcotoxin 1 family or homolog thereof; and regenerating the transformed plant cell into a transgenic plant expressing the member of the sarcotoxin 1 family or homolog thereof, wherein the DNA encoding the member of the sarcotoxin 1 family or homolog thereof is in an expression vector, said expression vector comprising an expression cassette comprising a first plant promoter induced by stress and a second plant promoter which is constitutively expressed, wherein the first plant promoter and the second plant promoter are positioned adjacent to each other, and wherein the

transgenic plant has enhanced resistance to pathogenic fungi as compared to a corresponding untransformed plant.

Applicants teach methods of constructing a recombinant gene and an expression cassette (Examples 1 and 2), methods of transforming a plant cell (Example 3), generating plants with enhanced resistant to pathogenic fungi compared to untransformed plants (Examples 5-6, and 8-10).

As discussed in the previous response filed February 2, 2001 and including the references cited and enclosed therein, the sarcotoxin 1 family of peptides and homologs thereof were well known in the art prior to filing the above referenced application. The sarcotoxin 1 family of peptides and homologs thereof include but are not limited to sarcotoxins 1a, 1b, 1c, 1d, and the cecropins. These peptides are short and nucleic acids encoding these peptides were also well known to those of skill in the art prior to filing the above referenced application. For example, Matsumoto *et al.*, *Biochem. J.* 239: 717-722 (1986) describe the cloning of and report the nucleotide sequence encoding the sarcotoxin 1a peptide from *Sarcophaga peregrina* (see Figure 1, page 719). Kanai *et al.*, *FEBS Lett.* 258: 199-202 (1989) describe the cloning of a gene cluster encoding the sarcotoxin 1 family and report the nucleotide sequence encoding sarcotoxin 1b from *Sarcophaga peregrina* (see Figure 1, page 200 and Figure 5, page 201). Okada *et al.*, *J. Biological Chem.* 260: 7174-7177 (1985) cited in the February 2 response, disclosed that the sarcotoxin 1c amino acid sequence differed from the sarcotoxin 1a amino acid sequence by only two amino acids (see Figure 5, page 7176). One of skill in the art using the known genetic code would be able to generate a nucleotide sequence encoding the sarcotoxin 1c peptide based on the sarcotoxin 1a nucleotide sequence using known PCR mutagenesis techniques.

The Examiner admits on page 3 of the office action, that references cited previously provide written description of cecropin nucleic acids from *Drosophila* and *Ceratitis* species. The Examiner also states on page 3 of the office action "[w]hereas Applicant need not disclose all of the species encompassed by the claimed genus, Applicant must describe a representative number of species." Applicants submit that they have provided adequate written description of a representative number of nucleic acid molecules encoding the sarcotoxin 1 family of peptides and homologs thereof as currently claimed.

Furthermore, those of skill in the art would know the metes and bounds of the term homolog as used in reference to sarcotoxin 1 based on the teachings of Lee *et al.*, *Proc. Nat. Acad. Sci USA* 86: 9159-9162 (1989). Lee *et al.*, describe a newly isolated peptide, termed cecropin P1,

which had amino acid sequence similarity ranging from 64% to 75% with sarcotoxin (cecropin) 1a and cecropin B respectively (see Lee *et al.*, page 9160, bottom of left column, right column and Figure 2). As discussed in Lee *et al.*, purified cecropin P1 had identical bactericidal activity as cecropin when measured in an anti-Ec (*E. coli*) assay (see Lee *et al.*, page 9159, Materials and Methods and page 9160 left column, first full paragraph). Based on this disclosure, one of skill in the art would know that the term homolog of the sarcotoxin 1 family member would have at least 64% to 75% amino acid similarity to sarcotoxin 1a and similar bactericidal activity when measured in an antibacterial assay.

In view of the above remarks, Applicants submit that they have provided an adequate written description of a sufficient number of species of the genus as currently claimed and that Applicants were in possession of the claimed invention at the time the application was filed. Applicants respectfully request that the 35 U.S.C. §112, first paragraph rejection of currently pending claims be withdrawn.

**35 U.S.C. §112, first paragraph, enablement:**

Claims 21, 22, 24-33, and 35-47 were rejected under 35 U.S.C. §112, first paragraph on the grounds that the specification is enabling only for claims limited to methods of enhancing fungal resistance with, and transgenic plants comprising, the sarcotoxin 1a gene. In particular, the Examiner states Applicants have not provided evidence that the structural relatedness of the sarcotoxin, and cecropin peptides corresponds to functional relatedness and that a cecropin peptide can be substituted for *e.g.* a sarcotoxin peptide. The Examiner further states that undo experimentation would be required to produce the nucleic acid molecules encoding the sarcotoxin family of peptides. Applicants respectfully disagree.

The Examiner is reminded:

Enablement is a legal determination of whether a patent enables one skilled in the art to make and use the claimed invention, *Raytheon Co. v. Roper Corp.*, 724 F.2d 951, 960 (Fed. Cir. 1983), is not precluded even if some experimentation is necessary, although the amount of experimentation needed must not be unduly extensive, *Atlas Powder Co. v. E.I. Du Pont De Nemours & Co.*, 750 F.2d 1569, 1576 (Fed. Cir. 1984), and is determined as of the filing date of the patent application. . . See *W.L. Gore and Associates v. Garlock, Inc.*, 721 F.2d 1540, 1556 (Fed. Cir. 1983). Furthermore, a patent need not teach and preferably omits, what is well known in the art. *Lindemann*, 730 F.2d at 1463, *Hybritech v. Monoclonal Antibodies, Inc.* 802 F.2d 1367, 1374 (Fed. Cir. 1986).

As the Examiner admits, the specification is enabling for methods of enhancing fungal resistance with, and transgenic plants comprising the sarcotoxin 1a gene. As discussed above, Applicants amend claims 21, 23, 25, 26, 30, 32, 34-36 and 39 herein to replace "anti-bacterial peptide from the Diptera insect" with "member of the sarcotoxin 1 family or homolog thereof." Also discussed above, Applicants submit herewith, references containing sequence information indicating that nucleic acid sequences encoding members of the sarcotoxin 1 family or homologs thereof were well known to those of skill in the art prior to filing the above referenced application or could be obtained without undue experimentation.

Applicants enclose, herewith, additional references indicating that the structural similarity among the sarcotoxin 1 family members and homologs thereof, relate to functional similarities as well. Iwai *et al.*, *Eur. J. Biochem.* 217: 639-644 (1993) disclose the NMR structure of sarcotoxin 1a. Data presented indicate that sarcotoxin 1a consists of 2 amphiphilic  $\alpha$ -helices (see Figures 1, 5, and 6 and page 643, right column, second, third and fifth full paragraphs), which is suggested to be important for penetrating the bacterial membrane. Iwai *et al.*, cite to a similar structural analysis of cecropin A, which indicates that cecropin A also contains two helical regions located in the same portions of the peptide as the sarcotoxin 1a helices (see page 643 right column, fifth paragraph and Figure 7). Iwai *et al.*, suggest that the structure of cecropin B is similar to cecropin A.

Other references indicate that the cecropins, like the sarcotoxins, also have a fungicidal activity. For example, DeLucca *et al.*, *Antimicrobial Agents and Chemotherapy* 41: 481-483 (1997), cited in the February 2, 2001 response, reported that cecropin A had fungicidal activity against both *Aspergillus* and *Fusarium* species (see page 482, left column and Figures 1 and 2). DeLucca *et al.*, *Medical Mycology* 36: 291-298 (1998) further report that cecropin B also had fungicidal activity against both *Aspergillus* and *Fusarium* species (see page 293, right column, page 294, Figure 1, and page 295, Discussion, paragraphs 1-3). Ekengren *et al.*, *Insect Biochemistry and Molecular Biology* 29: 965-972 (1999) report that cecropin A from *Drosophila* or *Hyalophora* and cecropin B from *Drosophila* were fungicidal against a number of different fungi (see page 967, paragraph 3.1 and Figure 1 and page 968, left column end of continued paragraph, right column beginning of Discussion and Table 1).

As discussed above, nucleic acids encoding the sarcotoxin 1 family and homologs thereof were also known or easily generated using known techniques by those of skill in the art the

time the application was filed (see *e.g.* Matsumoto *et al.*, *Biochem. J.* 239, 717-722 (1986), Kanai *et al.*, *FEBS Lett.* 258: 199-202 (1989) and previously cited Figure 2 of Kylsten *et al.*, *The EMBO J.* 9: 217-224 (1990), and Figure 1 of Rosetto *et al.*, *Gene* :134: 241-243 (1993)). For example, one of skill in the art could easily modify the known nucleic acid sequence of sarcotoxin 1a to produce a nucleic acid sequence encoding sarcotoxin 1c or 1d based on the known genetic code and the ability to synthesize long oligonucleotides for use in PCR reactions. Because the nucleotide sequences and/or amino acid sequences of various sarcotoxin family members are known, one of skill would not need to screen through a vast array of degenerate nucleic acid molecules, as the Examiner suggests, to generate nucleotide sequences encoding a sarcotoxin 1 family member or homolog thereof. As stated above, “[f]urthermore, a patent need not teach and preferably omits, what is well known in the art.” *Id.*

The Examiner suggests that the application is not enabled because Florack *et al.*, *Transgenic Research* 4: 132-141 (1995) report constructing a transgenic tobacco plant containing cecropin B which failed to confer resistance to pathogenic bacteria. In discussing the Florack *et al.*, article in their response filed December 28, 1999, Applicants distinguished the instant application from Florack stating in the first full paragraph on page 10 of the response “the plant expression system used by Florack *et al.*, was probably not efficient enough to express an amount of peptide necessary to confer resistance to pathogenic bacteria on the plant.” Applicants describe a dual promoter system on page 9, lines 5-17 of the specification and claimed in claim 21, lines 8-10 wherein both a constitutively expressed promoter and a promoter induced by stress control the expression of the sarcotoxin 1 family member or homolog thereof. Florack *et al.*, reported using only a constitutively expressed promoter to drive expression of cecropin B in their transgenic tobacco plants. Moreover, Applicants provide working Examples 5 and 6 and Figures 10 and 11 showing that the expression system used in the instant application is able to produce a sufficient amount of peptide in a transformed plant to convey enhanced resistance to pathogenic fungi compared to a corresponding untransformed plant as claimed. As stated in §2164.03 of the MPEP:

For a claimed genus, representative examples together with a statement applicable to the genus as a whole will ordinarily be sufficient if one skilled in the art (in view of level of skill, state of the art and the information in the specification) would expect the claimed genus could be used in that manner without undue experimentation. Proof of enablement will be required for other members of the claimed genus only where **adequate reasons** are advanced by the examiner to establish that a person

skilled in the art could not use the genus as a whole without undue experimentation. [emphasis added]

Due to the differences in the expression systems described in the instant application and Florack *et al.*, and the presence of working examples, Applicants submit that the Examiner has not advanced adequate reasons to establish that a person skilled in the art could not use the genus as a whole without undue experimentation.

In view of the above remarks, Applicants submit that the claims are fully enabled and respectfully request that the §112, first paragraph rejection of the currently pending claims be withdrawn.

**35 U.S.C. §112, second paragraph, indefiniteness:**

Claims 21-47 were rejected under 35, U.S.C. §112, second paragraph as allegedly indefinite. In particular, claims 21 and 32 were rejected on the grounds that the phrase “compared to the plant before transformation” in lines 6-7 or 7 respectively is unclear because the plant did not exist prior to transformation. Applicants have adopted the Examiner’s suggestion and have amended each claim to recite --compared to a corresponding untransformed plant--. Applicants respectfully request that the §112, second paragraph rejection of these claims be withdrawn.

Claim 25 was rejected on the grounds that the word “chitinasa” was misspelled. Applicants have adopted the Examiner’s suggestion and amended the claim to correct the spelling error. Applicants respectfully request that the §112, second paragraph rejection of this claim be withdrawn.

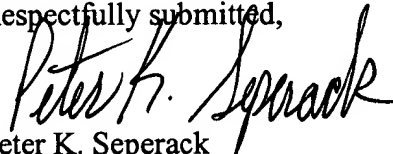
Claims 42-47 were rejected as allegedly indefinite for containing an improper Markush group and for lacking antecedent basis for the term “Diptera insect”. Claims 42, 43, 45, and 46 were rejected because the term homolog is allegedly indefinite. Claims 42 and 45 were rejected because the phrase “derived from” is indefinite. Applicants have canceled claims 42-47 herein and respectfully request that the §112, second paragraph rejection of these claims be withdrawn.

Claims 22-24, 26-31 and 33-41 were also rejected although the Examiner did not specifically enumerate a reason for the rejection. Applicants respectfully request that the §112, second paragraph rejection of these claims be withdrawn.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance and an action to that end is urged. If the Examiner believes a telephone conference would aid in the prosecution of this case in any way, please call the undersigned at 415-576-0200.

Respectfully submitted,



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**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

1                   21. (Amended) A method of conferring resistance to pathogenic fungi on a plant  
2 using a DNA sequence encoding [an anti-bacterial peptide from a Diptera insect] a member of the  
3 sarcotoxin 1 family or homolog thereof, the method comprising the steps of: transforming a plant  
4 cell by introducing the DNA sequence encoding the [anti-bacterial peptide from the Diptera insect]  
5 member of the sarcotoxin 1 family or homolog thereof; and regenerating the transformed plant cell  
6 into a transgenic plant expressing the [anti-bacterial peptide] member of the sarcotoxin 1 family or  
7 homolog thereof, wherein the DNA sequence encoding the member of the sarcotoxin 1 family or  
8 homolog thereof is in an expression vector, said expression vector comprising an expression cassette  
9 comprising a first plant promoter induced by stress and a second plant promoter which is  
10 constitutively expressed, wherein the first plant promoter and the second plant promoter are  
11 positioned adjacent to each other, and wherein the transgenic plant has enhanced resistance to  
12 pathogenic fungi as compared to [the plant before transformation] a corresponding untransformed  
13 plant.

1                   23. (Amended) The method according to claim 21, wherein the [anti-bacterial  
2 peptide from the Diptera insect] member of the sarcotoxin 1 family or homolog thereof is sarcotoxin  
3 1a.

1                   24. (Amended) The method according to claim 21, wherein the [DNA sequence  
2 encoding the anti-bacterial peptide from the Diptera insect is in an expression vector, said expression  
3 vector comprising an] expression cassette comprising the DNA sequence encoding the [anti-bacterial  
4 peptide from the Diptera insect] member of the sarcotoxin 1 family or homolog thereof is operably  
5 linked to [a] the first plant promoter and a drug resistance gene is operably linked to [a] the second  
6 plant promoter [which is constitutively expressed, wherein the first plant promoter and the second  
7 plant promoter are positioned adjacent to each other].

1                   25. (Amended) The method according to claim 21, wherein the DNA sequence  
2 encoding the [anti-bacterial peptide from the Diptera insect] member of the sarcotoxin 1 family or  
3 homolog thereof is operably linked to a plant gene via the hinge region of a tobacco [chitinase]  
4 chitinase gene.

1                   26. (Amended) The method according to claim 21, wherein the DNA sequence  
2 encoding the [anti-bacterial peptide from the Diptera insect] member of the sarcotoxin 1 family or  
3 homolog thereof is operably linked to a signal sequence from a plant gene.

1                   29. (Amended) The method according to claim [28] 21, wherein the promoter  
2 induced by stress is the promoter of the tobacco PR-1a gene.

1                   30. (Amended) The method according to claim 24, wherein the expression cassette  
2 further comprises the terminator of the tobacco PR-1a gene operably linked downstream of the DNA  
3 sequence encoding the [antibacterial peptide from the Diptera insect] member of the sarcotoxin 1  
4 family or homolog thereof.

1                   31. (Amended) The method according to claim [24] 21, wherein the second plant  
2 promoter is the cauliflower mosaic virus 35S promoter.

1                   32. (Amended) A plant which confers resistance to pathogenic fungi, the plant  
2 comprising an expression vector comprising an expression cassette comprising a DNA sequence  
3 encoding [an anti-bacterial peptide from a Diptera insect] a member of the sarcotoxin 1 family or  
4 homolog thereof operably linked to [an inducible] a promoter induced by stress and a drug resistance  
5 gene operably linked to a constitutively expressed promoter, wherein the [inducible] promoter  
6 induced by stress and the constitutively expressed promoter are positioned adjacent to each other,  
7 wherein the transgenic plant has enhanced resistance to pathogenic fungi as compared to [the plant  
8 before transformation] a corresponding untransformed plant.

1                   34. (Amended) The plant according to claim 32, wherein the [anti-bacterial peptide  
2 from the Diptera insect] member of the sarcotoxin 1 family or homolog thereof is sarcotoxin 1a.

1                   35. (Amended) The plant according to claim 32, wherein the DNA sequence  
2 encoding the [anti-bacterial peptide from the Diptera insect] member of the sarcotoxin 1 family or  
3 homolog thereof is operably linked to a plant gene via the hinge region of a tobacco chitinase gene.

1                   36. (Amended) The plant according to claim 32, wherein the DNA sequence  
2 encoding the [anti-bacterial peptide from the Diptera insect] member of the sarcotoxin 1 family or  
3 homolog thereof is operably linked to a signal sequence from a plant gene.

1                   38. (Amended) The plant according to claim [37] 32, wherein the promoter induced  
2 by stress is the promoter of the tobacco PR-1a gene.

1                   39. (Amended) The plant according to claim 32, wherein the expression cassette  
2 further comprises the terminator of the tobacco PR-1a gene operably linked downstream of the DNA  
3 sequence encoding the [anti-bacterial peptide from the Diptera insect] member of the sarcotoxin 1  
4 family or homolog thereof.